# DEVELOPMENT OF A FIELDABLE, RAPID, ACCURATE & SENSITIVE BIO-ELECTRONIC, DNA BIOSENSOR

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## 1. OVERVIEW

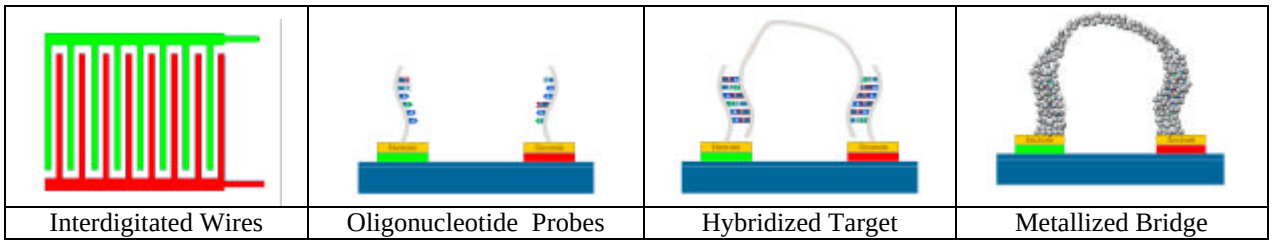
Integrated Nano-Technologies (INT) has developed a novel electronic biosensor technology that will rapidly and accurately provide DNA-based field detection and identification of pathogenic organisms. The sensor is capable of detecting the binding of a single molecule of DNA or RNA and therefore does not require the use of PCR. The sensor chip can be engineered with an array of hundreds of independent test sites, which allows for confirmatory tests leading to a high level of specificity, the ability to screen for multiple threat agents simultaneously, and the capability to detect genetically engineered organisms.

Biological agents continue to pose a major threat to U.S. troops as well as U.S. civilians both domestically and overseas. Rapid, accurate and sensitive detection and identification of biological agents is crucial to effective containment, neutralization and/or treatment. The U.S military has an immediate need for a rapid, field-deployable system for the detection and identification of biological agents, not only to protect military personnel from their effects, but also to contain and destroy them. An effective system must be: accurate, sensitive, rapid, easy to use, portable and field-ready. None of the currently available technologies meet all the necessary criteria.

The most specific and accurate method of identifying a biological agent is by targeting its unique DNA. Currently, Polymerase Chain Reaction (PCR) amplification followed by fluorescent analysis is the most common method of DNA identification. PCR is a well-understood and reliable laboratory process, but it is highly susceptible to contamination, is labor intensive, and requires a skilled operator and specialized equipment. It is best suited to use within a laboratory or other controlled environment. Attempts to deploy PCR to field environments have proven largely ineffective.

The resulting challenge has been to develop a new technology that provides the accuracy of PCR, and can be easily taken into the field. Since its introduction in 1985, PCR has been the standard by which DNA based assays are judged. Because PCR was such a substantial discovery and because it has allowed for countless scientific advances, efforts to achieve a rapid, accurate portable DNA identification system have been primarily focused on extending and simplifying PCR. These efforts have yielded limited success.

INT's biosensor is unique in its use of a combination of a biological event (DNA hybridization) with a chemical event (metal development) and microelectronics, to electronically produce a strong electrical signal that indicates the presence of an organism-specific target DNA. The biosensor consists of oligonucleotide probes attached to multiple pairs of interdigitated electrodes on a microchip. Biological samples are processed to produce a solution of DNA fragments that are passed over the sensor's surface. Hybridization of a target DNA to the DNA capture probes bound to the electrodes forms a DNA bridge connecting the two electrodes. A chemical treatment of this DNA bridge coats it with metal, converting it to a conductive wire. The sensor is then electrically analyzed to determine if any bridges have formed. When as little as one bridge is formed and metallized, the electrical resistance of the sensor is reduced more than 1000X. The core technology of the INT biosensor is illustrated below in Figure 1.



| Interdigitated Wires | Oligonucleotide Probes | Hybridized Target | Metallized Bridge |
|---|---|---|---|

Figure 1 – INT's Biosensor Process

# Report Documentation Page

*Form Approved*
*OMB No. 0704-0188*



| 1. REPORT DATE | 2. REPORT TYPE | 3. DATES COVERED |
|---|---|---|
| **00 DEC 2004** | **N/A** | **-** |

| 4. TITLE AND SUBTITLE | 5a. CONTRACT NUMBER |
|---|---|
| **Development Of A Fieldable, Rapid, Accurate & Sensitive Bio-Electronic, Dna Biosensor** | **g** |
| | 5b. GRANT NUMBER |
| | 5c. PROGRAM ELEMENT NUMBER |
| 6. AUTHOR(S) | 5d. PROJECT NUMBER |
| | 5e. TASK NUMBER |
| | 5f. WORK UNIT NUMBER |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Integrated Nano-Technologies Henrietta, NY 14467** | 8. PERFORMING ORGANIZATION REPORT NUMBER |
| 9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) | 10. SPONSOR/MONITOR'S ACRONYM(S) |
| | 11. SPONSOR/MONITOR'S REPORT NUMBER(S) |

12. DISTRIBUTION/AVAILABILITY STATEMENT
**Approved for public release, distribution unlimited**

13. SUPPLEMENTARY NOTES
**See also ADM001736, Proceedings for the Army Science Conference (24th) Held on 29 November - 2 December 2005 in Orlando, Florida., The original document contains color images.**

14. ABSTRACT

15. SUBJECT TERMS

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | |
| **unclassified** | **unclassified** | **unclassified** | **UU** | **4** | |

| INT Technology | Systems Requiring PCR |
|---|---|
| *Ease-of-use/Low Cost* | *Complex/Expensive* |
| *Rapid*: | *Slow* |
| *Sensitive.* | *Imprecise* |
| *Minimal Sample Preparation* | *Subject to Contamination* |
| *Lightweight and Portable* | *Heavy and Cumbersome* |
| *Multiplexing* | *Limited Multiplexing* |

Figure 2. – INT vs. PCR

INT's sensors have 64 independent test sites. The next generation chip is being designed which will allow for 256 simultaneous tests and the ability to quantitate the amount of DNA in a sample.

INT's biosensor is fundamentally different from all other technologies currently available and will provide significant improvements in sensitivity, speed, portability, ease-of-use, and cost. Furthermore, INT's detection system uses a streamlined sample prep procedure which can be readily integrated into systems with air-sample collectors and reporting and data storage technology. The U.S. Army could benefit greatly from this technology.

This paper will describe in detail, INT's research and biosensor development efforts to date and will highlight future applications of INT's technology in this arena and beyond.

## 2. THE BIODETECT PLATFORM

BioDetect uses a series of simple steps and two components to achieve the desired results. They are:

### A. Hybridization:

Once prepared, the DNA is introduced to the microchip surface containing capture probes complementary to a DNA sequence of interest. Hybridization occurs with a high degree of specificity because (1) two complementary binding events are required (i.e., one to each electrode) and (2) the DNA fragment must be of sufficient length to span the inter-electrode gap.

### B. Metallization:

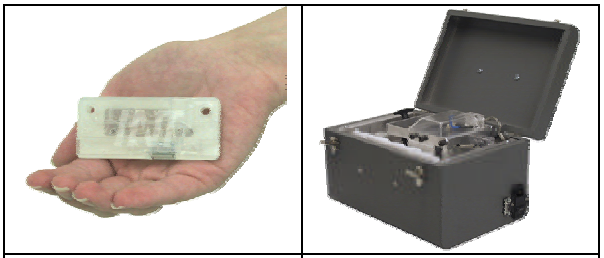
DNA by itself is not electrically conductive, therefore after hybridization the DNA must be made conductive. The DNA is used as a substrate for the deposition of metal and construction of a nanowire. The decreased resistance of test structures with metallized DNA bridges indicates the presence of a target DNA. Several metallization chemistries have been developed for use with the biosensor focusing on the ideal balance of: rapid reaction time, minimal background, and no adverse effect on hybridization.

### C. Electronic Detection:

The final step in the detection process is to measure the electrical resistance of each of the test structures. Voltage is applied to one of the two electrodes in each test structure and the resistance is obtained by probing the opposite electrode. INT has observed that a single DNA bridge formation results in at least a 1,000 fold reduction in resistance on the test structure.

### D. Components

The BioDetect system is built around a two component design: an inexpensive, self-contained disposable test cartridge and an analyzer into which the cartridge is loaded for testing. This two-component approach provides maximum configuration flexibility, while keeping the system inexpensive to operate and minimizing disposables.



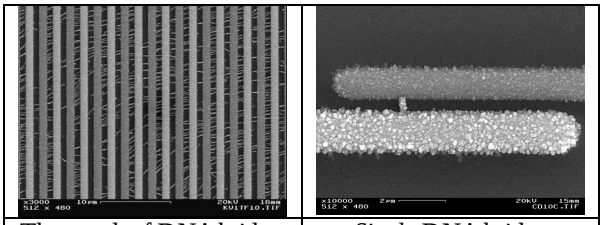
| BioDetect Test Card | BioDetect Analyzer |
|---|---|

Figure 3. – BioDetect Components

Within each test cartridge, there is a simple silicon chip with multiple independently addressable test structures arrayed upon it. Current chip architecture supports 64 test structures, each of which may test for the same or multiple target organisms simultaneously. Each independently addressable test structures measures about 400 µm square. The generation II chip, which is in development, will have 250+ independently addressable test structures with embedded logic that will permit quantitation assays in addition to identification.

Scanning Electron Micrographs ("SEM") of BioDetect sensors after metallization reveal the conductive nanowires formed between electrodes when a target biological is present. The figures on the left show a test structure of target DNA hybridized to capture probes (top) and a magnified view of a single wire (20-40 nm in diameter) is shown in Figure 4 below.

INT has successfully demonstrated electronic detection of gene targets from samples of genomic DNA from *Bacillus subtilis*. No amplification of the target sequences is performed before detection. In addition, INT



Thousands of DNA bridges on a BioDetect Sensor | Single DNA bridge

Figure 4. – SEM Images

has developed test chips targeted to various sequences from *Bacillus anthracis* genes. Current research involves an expanding list of pathogens.

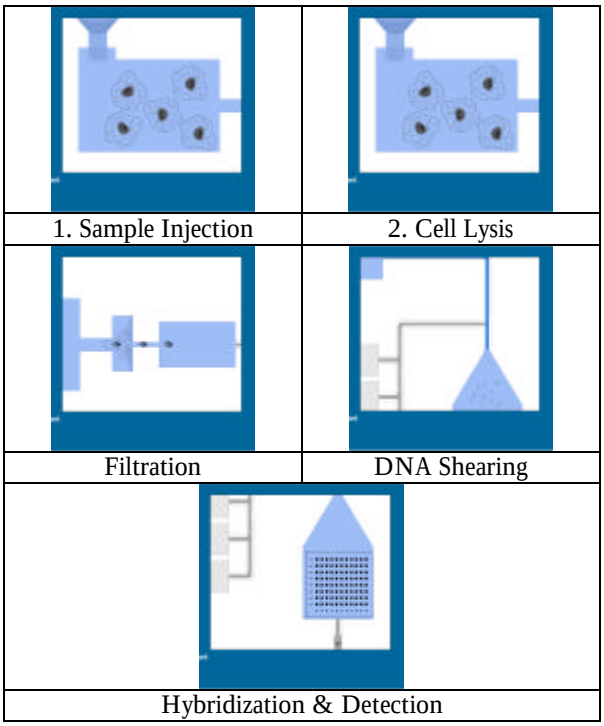
## 3 SAMPLE PREPARATION

Sample preparation requires efficient release of DNA from a sample, filtering of the sample, and breaking the DNA down to a size appropriate for the sensor. Because the BioDetect system does not require amplification of the target nucleic acid molecules, sample prep and processing requirements can be incorporated into a simple, automated procedure. Most inhibitors of the enzymes required for DNA amplification will not have an effect on the process utilized. Additionally, detergents and organic solvents can be used to decrease non-specific binding and inhibit degradation of target nucleic acid molecules, especially RNA targets.

There are several effective methods for releasing DNA from cells, including chemical lysis and sonication. Chemical lysis works well for human cells, common bacteria, and viruses. Sonication is more effective for disrupting bacterial spores. For most samples, chemical disruption is sufficient.

After lysing, the sample is filtered to remove anything in the sample that could aberrantly short the sensors. Again, because the system does not use enzymes, it is not necessary to remove all chemical contaminants. Furthermore, the nucleic acid analog probes are not affected by salt concentrations in the sample.

The released DNA must be sheared to a length that works with the BioDetect sensor. Current DNA fragment size is between 1,000 and 6,000 base pairs in length. Future chip designs will lower the required target DNA length to several hundred base pairs. INT has identified a shearing method which provides fragments with within the desired ranges. This method involves pushing DNA through a small bore opening into a larger bore vessel. The average length can be controlled by changes to flow rate and the size of the opening. The resulting fragments fall within a 2-fold size distribution.



1. Sample Injection | 2. Cell Lysis

Filtration | DNA Shearing

Hybridization & Detection

Figure 5 – Sample Prep Process

The sheared DNA is then moved into the hybridization chamber where it can bind to the test sites on the sensor. The DNA is manipulated by mechanical mixing, electrical fields and pulsing of the fluids. After hybridization, all unbound DNA is washed into a waste chamber.

## 4. CONCLUSIONS

In summary, INT's system requires minimal sample prep. The process which involves cell lysis, DNA shearing and filtering can be accomplished in a single pass through cartridge which can be integrated with the detection sensor to produce a fully automated system.

The system can be highly multiplexed to test for numerous biological agents simultaneously, to provide confirmatory tests for different unique sequences from a target organism, and to provide highly accurate and quantitative results.

The new design under development will incorporate CMOS-based logic. This will allow the BioDetect system to produce highly multiplexed results quickly and inexpensively using a combination of on-chip logic, statistics and bioinformatics. The logic chips will have 256 separately addressable test sites. Future chips may have several thousands of test sites with each site possessing a unique set of probes. Additionally, each test site will be subdivided into thousands of subsensors,

allowing for the collection of data for statistical and quantitative analysis. The data will be analyzed using algorithms which will weigh results from the various sensors and calculate the statistical level of certainty of a positive or negative result and provide quantitative results. Error recognition software will be utilized to recognize patterns from handling damage to electrical signals from debris, further increasing the reliability of the system.

Due to the ability to multiplex, the system can provide for more information regarding an agent than simply a yes or no identification. Through the proper design of probe sets, BioDetect chips can identify genetically altered organisms, determine drug resistances, and even provide a taxonomic analysis of an unknown organism.

BioDetect is a significant advance over current technologies. It has been described by knowledgeable commentators as "disruptive technology". BioDetect can match or exceed the sensitivity and accuracy of PCR-based assays in the field while delivering the speed, portability and ease-of-use of much simpler ELISA-type assays.

BioDetect technology is well suited for deployment in the field for rapid non-PCR analysis of biologicals. Its open architecture permits retrofitting or integration with legacy systems. Accuracy, multiplexing, and the ability to operate with a variety of environmental contaminants, make BioDetect a logical choice for field systems. BioDetect is also simpler and much less expensive to operate than systems relying on PCR. As a result, there is an opportunity for significant cost savings by replacing PCR-based detection with BioDetect.

The US Army could benefit from BioDetect technology by using it in the following ways:

Force Protection

- Man-portable, vehicle/UAV-mounted units for point detection
- Fixed installation units for perimeter detection and sensor-nets
- Field-diagnostics for identifying Force exposure
- Follow-up diagnostics for gauging treatment effectiveness

Biological WMD Identification

- Rapid, accurate substance assessment in the field
- Identification of modified/engineered organisms

Public Safety

- Rapid, accurate substance assessment in the field
- Decision-reliable results on-site, minimizing panic and public impact from false alarms

INT has successfully demonstrated electronic detection of gene targets from samples of genomic DNA *Bacillus subtilis*. No amplification of the target sequences is performed before detection. In addition, INT has developed test chips targeted to sequences from lef and pagA genes from *Bacillus anthracis*. Current research is focused on improvements to specificity, sensitivity and automation.